Video Article

Microinjection of A. aegypti Embryos to Obtain Transgenic Mosquitoes

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Abstract

In this video, Nijole Jasinskiene demonstrates the methodology employed to generate transgenic Aedes aegypti mosquitoes, which are vectors for dengue fever. The techniques for correctly preparing microinjection needles, dessicating embryos, and performing microinjection are demonstrated.

Protocol

Preparation in advance of microinjection

1. Blood feed mosquitoes: for injection from Monday to Friday feed females on the previous Thursday.
2. Prepare glass needles using program 3 or 2 (see Materials).
3. Laying tube for the embryos is Drosophila culture vial. Wet cotton wool in the bottom, with a wet disk of filter paper covering it.
4. Prepare plastic cover slip by sticking double-side tape to one end. Trim tape to cover slip so that it ends at edge of cover slip.
5. Prepare oil for desiccation (see Materials)
6. 150 ml plastic beaker with a wet cotton wool in the bottom, covered with a wet disk of filter paper (80mm Whatman) to keep embryos after injection.

Set up of forced laying

1. Collect 6-10 blood fed females with the use of an aspirator and transfer them to the Drosophila culture vial with wet cotton and filter paper.
2. Put the mosquitoes back into insectory conditions in the dark, and allow to lay eggs for 1h and 15min.
3. Let adults fly into the cage and remove the filter paper disk with embryos.
4. To line up eggs, do so under dissecting scope. Using fine forceps No. 5 or a fine paintbrush, pick gray to darkish-grey embryos and arrange in line on square of 3MM Whatman paper soaked with water. All embryos must be in the same orientation, as injection has to be at the posterior pole. The anterior end of the embryo is slightly wider than the posterior. Dry the filter paper with embryos by pressing dry filter paper to it (wet things won't stick to tape). To transfer the eggs, invert the slide containing the double-sided tape and gently press against the eggs. The posterior end of the eggs have to be very close to the edge of the double sided tape.
5. Desiccate the embryos about 1 min. at room temperature. They start to dimple slightly as they dry.
6. Cover desiccated embryos with halocarbon oil to prevent further desiccation.

Microinjection of the embryos

1. The most important aspect of good injection is the quality of the needle (see Materials).
2. Fill a needle with the DNA solution to be injected by using a microloader. Very little injection solution is needed: 1 to 2 µl.
3. Connect the needle to the Transjector, which controls the injection time (Manu) and pressure (600), and the backpressure (250). Microinjection is performed using a microscope with a moving stage (Leica) at x 10 magnification and micromanipulator. If necessary, a raised microscope stage may be prepare by stacking 4-5 microscope slides. Place the cover slip carrying the embryos onto raised stage. Inject embryos horizontally to prevent tearing of the endochorion; penetration should be at the posterior pole. For injection, I keep the needle stationary and move the stage of the microscope.
4. Inject 0.2-0.5 nl of solution, corresponding to approximately 1-5% of the embryo volume. Control the injection volume by adjusting the injection pressure and time. The slightly bowed, desiccated embryos should regain their turgid appearance following injection.
5. Pick embryos from the cover slip using fine forceps or a fine brush, and place them in a plastic beaker with wet cotton and wet filter paper. Cover beaker with tissue paper. Keep in place with elastic band around the rim and return to insectary conditions for 4 days.

Post-injection

1. Four days after, carefully place embryo paper, embryo-side down, on the water in large plastic box with a small amount of food. Embryos hatch over a very long period: don't throw out the culture until at least 4-5 weeks post-injection.

Notes

DNA for injection

Plasmid DNA solution for injection was isolated using EndoFree plasmid kit. Construct and Helper plasmid mix together in right concentration, precipitated with izopropanol. The pellet was washed with 70% ethanol before resuspending in injection buffer. Before injection, clean DNA using Millex-GV column.

References